

**REMARKS****I. Status of the Claims**

Claims 1-13 are pending in the application. Claims 1-13 are rejected. Applicants respectfully request reconsideration of the claims in view of the following remarks.

**II. Claims 1-13 Meet the Requirements of 35 U.S.C. § 112, Second Paragraph**

Claims 1-13 are rejected under § 112, second paragraph with respect to the recited amounts of calcium chloride and with respect to the amount of calcium in the modified Earle's. Claims 12 and 13 are rejected under § 112, second paragraph as not indicating what volume is intended for ascorbic acid. Applicants respectfully traverse the rejection.

Claims 1 and 13 are each directed to isolation of adult cardiac cells. The methods defined by claims 1 and 13 can aid in obtaining viable adult cardiac myocytes and promoting long-term survival of adult cardiac myocytes. See page 4, line 31 to page 5, line 6 of the instant application. Each of claims 1 and 13 recites, in part, "successively exposing the tissue to a first solution with amount of calcium chloride decreasing from about 1-2  $\mu$ M." This step clearly refers to exposing tissue to a first solution with a selected amount of calcium chloride and then exposing the tissue to an additional solution having an amount of calcium chloride that is about 1-2  $\mu$ M less than the selected amount of calcium chloride in the first solution. After disassociation of the tissue with an enzyme solution, the disassociated tissue is first resuspended in a second solution with a selected amount of calcium chloride and then repeatedly resuspended in at least one additional solution that has an amount of calcium chloride that is about 1-2  $\mu$ M more than the selected amount of calcium chloride in the second solution. In view of the above, the steps of claims 1 and 13 that recite the use of solutions with "decreasing" and/or "increasing" amounts of calcium chloride are clear and definite, and claims 1 and 13 meet the requirements of 35 U.S.C § 112, second paragraph. Each of claims 2-12 depends either directly or indirectly from claim 1 and meets the requirement of 35 U.S.C § 112, second paragraph for at least the same reasons as claim 1.

With respect to the amount of calcium in the modified Earle's, it is within the ability of the person of ordinary skill in the art, given the benefit of the instant specification, to select amounts of calcium chloride as starting and final points. It should be noted that claims 1 and 13

are not limited to any particular starting or final amounts of calcium chloride, but instead recite the use of decreasing or increasing amounts of calcium chloride based on an initial selected amount of calcium chloride. Accordingly, claims 1-13 meet the requirements of 35 U.S.C § 112, second paragraph.

With respect to the lack of volume for ascorbic acid, as recited in claims 12 and 13, there is no requirement under 35 U.S.C § 112, second paragraph to define the amount of a reagent in terms of concentration. Because the specified amount of ascorbic acid is presented in standard terms of weight and measure, it is within the ability of the person of ordinary skill in the art, given the benefit of the instant application, to make any adjustments to reach a desired volume. Accordingly, claims 12 and 13 meet the requirements of 35 U.S.C § 112, second paragraph.

Applicants respectfully request withdrawal of the rejection.

**III. Claims 1-13 are Patentable over Kruppenbacher  
in combination with the ATCC Catalog and Kang**

Claims 1-13 are rejected under § 103(a) over Kruppenbacher (Naturwissenschaften 80, 132-134 (1993)) in combination with the ATCC catalog (page 522) and Kang (P.N.A.S. 91, 9886-9890 (1994)). Applicants respectfully traverse the rejection.

Claims 1-13 are patentable over the combination of Kruppenbacher, ATCC catalog and Kang, because the combination of these citations fails to teach or suggest the subject matter defined by any of claims 1-13. Instead, Kruppenbacher is directed to a method of isolating cardiac myocytes that uses only increasing amounts of calcium chloride. Kruppenbacher places hearts in a perfusion buffer that includes 25  $\mu$ M calcium chloride. See left column of page 133. These hearts are then cut into pieces and chopped in the same perfusion buffer. The resulting tissue suspension is incubated in a water bath, but nowhere does the method of Kruppenbacher expose the tissue suspension to water as asserted by the Examiner. After incubation in the water bath, the cardiomyocytes are recalcified in three steps using 0.2, 0.5 and 1 mM calcium chloride. The method of Kruppenbacher thus exposes cardiomyocytes to a solution with 25  $\mu$ M calcium chloride and then exposes the cardiomyocytes to solutions having 0.2, 0.5 and 1 mM calcium chloride. That is, the method of Kruppenbacher only exposes cardiomyocytes to increasing amounts of calcium chloride.

In contrast, the method defined by each of claims 1 and 13 successively exposes tissue to decreasing amounts of calcium chloride and then repeatedly suspends disassociated tissue in increasing amounts of calcium chloride. Kruppenbacher's failure to teach or suggest any exposure of tissue to successively decreasing amounts of calcium chloride leaves Kruppenbacher deficient and incapable of rendering any of claims 1-13 obvious.

The deficiencies of Kruppenbacher are not cured by the ATCC catalog or Kang. In particular, there is no teaching or suggestion in the ATCC catalog regarding the subject matter defined by claims 1-13. Instead, the ATCC catalog was cited for the sole purpose of the ingredients in M199 medium. Kang also fails to teach or suggest the use of decreasing amounts of calcium chloride to isolate adult cardiac cells. Kang uses a single perfusion buffer with a single concentration of calcium to measure contractility of neonatal rat cardiac myocytes. See paragraph bridging pages 9886-9887. Nowhere does Kang teach or suggest isolation of adult cardiac cells by successively exposing tissue to solutions of decreasing amounts of calcium chloride and then repeatedly resuspending disassociated tissue in solutions including increasing amounts of calcium chloride. Thus, neither the ATCC catalog nor Kang cure the deficiencies of Kruppenbacher.

Notwithstanding Kang's deficiencies discussed above, Kang also leads away from the subject matter of claims 1-13. Kang is directed to studies regarding contraction of neonatal rat cardiac myocytes, whereas claims 1-13 are directed to isolation of adult cardiac cells. Thus, Kang leads away from isolation of adult cardiac cells and cannot be properly applied to claims 1-13.

In addition, no suggestion or teaching has been identified that clearly indicates the person of ordinary skill in the art would look to either the ATCC catalog or Kang to cure any deficiencies in Kruppenbacher. It is impermissible to combine citations to establish obviousness when there is no suggestion or motivation to combine the citations.

In view of the above, claims 1-13 are each patentable over Kruppenbacher in combination with the ATCC catalog and/or Kang. Applicants respectfully request withdrawal of the rejection.

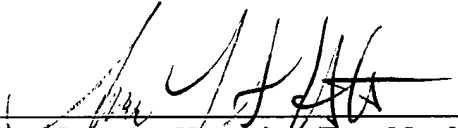
**IV. Conclusion**

As discussed above, claims 1-13 are patentable over all citations of record. A Notice of Allowance is respectfully requested.

Should the Examiner believe, after consideration of these remarks, that the application is not in condition for allowance, the Examiner is invited to call the Applicants' attorneys at the telephone number listed below.

If there is any fee occasioned by this response, please charge any deficiency to Deposit Account No. 50/2762 (Reference G2000-700210).

Respectfully submitted,  
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